1: Anal Biochem 1995 May 20;227(2):255-73

Revolutions in rapid amplification of cDNA ends: new strategies for polymerase chain reaction cloning of full-length cDNA ends.

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Rapid amplification of cDNA ends (RACE) is a polymerase chain reaction (PCR)-based technique which was developed to facilitate the cloning of full-length cDNA 5'- and 3'-ends after a partial cDNA sequence has been obtained by other methods. While RACE can yield complete sequences of cDNA ends in only a few days, the RACE procedure frequently results in the exclusive amplification of truncated cDNA ends, undermining efforts to generate full-length clones. Many investigators have suggested modifications to the RACE protocol to improve the effectiveness of the technique. Based on first-hand experience with RACE, a critical review of numerous published variations of the key steps in the RACE method is presented. Also included is a detailed, effective protocol based on RNA ligase-mediated RACE/reverse ligation-mediated PCR, as well as a demonstration of its utility.

Publication Types:
- Review
- Review, Tutorial

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1: J Virol 1993 May;67(5):2621-7

Concurrent sequence analysis of 5' and 3' RNA termini by intramolecular circularization reveals 5' nontemplated bases and 3' terminal heterogeneity for lymphocytic choriomeningitis virus mRNAs.

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We have used a technique of RNA circularization coupled with polymerase chain reaction amplification for simultaneous analysis of the 5' and 3' termini of subgenomic mRNAs derived from the S RNA of lymphocytic choriomeningitis virus during an acute infection of BHK cells. These mRNAs possess 1 to 7 nontemplated nucleotides of apparently random sequence at their 5' ends. The predominant mRNA species have 4 or 5 nontemplated nucleotides. The 5' termini of the mRNAs also have properties consistent with the presence of a 5' cap structure. The 3' termini of the mRNAs lack poly(A) tails, and we have shown that transcription termination occurs at heterogeneous positions within the intergenic region of the S RNA. The identification of several distinct termini in the vicinity of a putative stem-loop structure in the RNA templates suggests that transcription termination may be mediated by a structural signal rather than a precise sequence signal.

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1: Eur J Biochem 1984 Oct 15;144(2):261-9 Related Articles, NEW Books

Yeast mitochondria contain a linear RNA strand complementary to the circular intronic bI1 RNA of cytochrome b.

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bI1 RNA (excised from the first intron of the long form of the cytochrome b gene of Saccharomyces cerevisiae mitochondria) hybridizes with the two strands of a Bg/II-MboI DNA segment from this region. This fraction is resistant to digestions by DNase I and RNase T1 and disappears completely upon alkali hydrolysis. Strand-specific labeling of an intronic DNA fragment, cloned in pBR322 plasmid, was accomplished through the use of a T4 DNA polymerase. The purity of the probes was demonstrated by cloning an exon-intron fragment and labeling it by the same procedure; mRNA and pre-mRNA bands hybridized only with the transcribed DNA strand whereas bI1 RNA hybridized with the two strands under the stringent washing conditions employed (tm + 20 degrees C). Several experimental results argue against the possibility that the observation of two complementary bI1 RNA strands results from a partial self-complementarity of the RNA. A pre-mRNA intermediate from a box8 (G5046) mutant, still containing this intron, hybridizes only with the transcribed DNA strand of the pure intronic probe. The amount of the non-sense bI1 RNA strand is very low, in cells from two wild-type strains, relative to the sense RNA strand during the early stages of growth on glucose. It increases as the cells are released from glucose repression. bI1 RNA is resistant to RNase. Very little self-complementarity is seen by computer analysis of the sequence. Purified bI1 RNA is seen by electron microscopy under non-denaturing conditions as a mixture of double-stranded circular and linear molecules thus confirming the existence of the two complementary strands. The disappearance of all material following alkali hydrolysis demonstrates that these are indeed two RNA strands. Under fully denaturing conditions a mixture of single-stranded circular and linear molecules is seen as reported previously (Cell, 19, 321-329, 1980). We conclude that yeast mitochondria contain the two complementary bI1 RNA strands, one circular and the other linear. Considering a largely asymmetrical transcription of the mitochondrial genome in yeast and assuming that circularization of some intronic RNAs is part of RNA processing, we do not believe that the two strands are each a mixture of linear and circular molecules. The ratio of non-sense to sense bI1 RNA in a cytoplasmic petite mutant, A1B1, also varies according to growth conditions.(ABSTRACT TRUNCATED AT 400 WORDS)